Post-translational modifications (PTMs) in priming the plant immune system: ripe for exploitation?

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Abstract

Microbes constantly challenge plants and some can successfully infect their host and ultimately cause disease. In order to cope against pathogen infection, plants must be ready to "fight back". Basal immunity in many cases, is not enough for survival and leads to disease and ultimately a premature death of the host. However, the plant immune system can be temporarily and even trans-generationally primed; this 'primed state' leads to changes in the plant involving transcriptional, post-translational, metabolic, physiological and epigenetic reprogramming, which enables the plant to fine-tuning its defence mechanisms for a rapid and/or more robust response after abiotic and/or biotic stress. This can ultimately affect pathogen infection speed and hence decrease its ability to overcome host resistance and the final outcome of the host-pathogen interaction. The role of the three major PTMs (protein ubiquitination, phosphorylation and SUMOylation) in plant immunity has been well-established and new PTMs have emerged as plant cell signalling regulators such as S-acylation. However, the role of PTMs on defence priming and how PTM machinery is affected in primed plants and its connection to plant resistance against biotic/abiotic stress is not well understood. This review highlights the current state of play of priming-mediated post-translational reprogramming and explores new areas for future research.

Key words: basal immunity, gene-for-gene resistance, priming, defence, post-translational modifications, SUMOylation, epigenetics

Introduction

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INTRODUCTION

1. Plant Innate immunity: basal resistance and post-translational modifications

Unlike animal cells, plants depend on their innate immunity due to their lack of somatic adaptive defences [1]. However, plants are not unprotected against the pathogens and pests that attack them. They have a developed and sophisticated immune system that must be able to endure attacks from a wide variety of microorganisms, such as bacteria, oomycetes, fungi and viruses. Despite the fact that pathogens have different host ranges depending on their nature and specialization level; it is well-known they have coevolved with plants over millions of years [2, 3] to develop a way to infect them, and at the same time plants have developed more or less successful ways to resist infection and disease development. This co-evolutionary development of the plant immune system has been generally accepted and represented by a zig-zag model [1,4].

Many pathogens, such as oomycetes, aphids and fungi are able to penetrate directly their host cell wall, unlike plant viruses and bacteria, which depend on natural openings, damaged tissue or vectors [5]. In order to fight pathogen infection, plants have created a series of resistance mechanisms. As a first physical defence, plants have a waxy layer on their leaf surfaces beneath which are a series of cell-wall defences, such as lignin and callose appositions, so-called papillae. If a pathogen attempts to infect and subsequently cause disease in the plant it needs to first overcome these physical barriers. These callose-rich papilla depositions are usually induced ubiquitously in plants upon pathogen attack, in contrast with other types of defence pathways [6].

If a pathogen does manage to penetrate through these layers, the plant needs to be able to combat it. As a primary defence response, plants have a wide range of specific cell-wall surface receptor-type proteins called pattern-recognition receptors (PRRs) that respond to microbes through the sensitive and quick recognition of conserved microbial features [7], such as chitin, flagella, glycoproteins or lipopolysaccharides, called microbe-associated molecular patterns (MAMPs) and pathogen-associated molecular patterns (PAMPs), or molecules released on damaged tissue called damage-associated molecular patters (DAMPs) [5]. This recognition triggers a set of defence mechanisms in the plant that results in the activation of PAMP-triggered immunity (PTI) which can prevent the pathogen from infecting and colonising host tissues.

It has been discovered that successful pathogens have acquired host-specific molecules called effectors [8] that they release to prevent host recognition of their PAMPs/MAMPs or by directly suppressing PTI responses [9].

Peptide-based post-translational modifications (PTMs) are regulatory processes that can alter the function, structure and activity of the proteome. Studies on the role of PTMs in plant immunity and cell signalling have increased over the last decade [10]. Furthermore, the three major PTMs, protein phosphorylation, ubiquitination and SUMOylation, are well-known to mediate PTI and R gene-dependent signalling. PTI-induced mitogen-activated protein kinase (MAPK) signalling regulates transcription factors through phosphorylation which are in turn targets for the Small Ubiquitin-like Modifier (SUMO) protein [11]. Plasma membrane-related proteins are also a target for lipid-based post-translational modifications, including S-acylation, N-myristoylation, prenylation and glycosylphosphatidylinositol (GPI) anchors [12]. This review briefly examines some key aspects of the three major post-translational modifications (PTM) (ubiquitination, phosphorylation and SUMOylation) in plant immunity and defence priming with an aim to provide new insights into current knowledge.

In a constant plant-pathogen arms race, plants acquired a second layer of immune response in which they can recognise effectors with resistance (R) proteins and subsequently trigger so-called effector-triggered- immunity (ETI) [13]. This coevolution between the pathogen and the host, where the pathogen avirulence (Avr) gene evolves to avoid recognition and the host resistance (R) gene changes in order to scan and recognize pathogen MAMPS/PAMPS is accepted as the distinctive gene-for-gene model [3].

During this plant-pathogen interaction there is an onset of defence systems triggered by the plant which leads to resistance or, if ineffective, disease development. Many different R and Avr proteins have been characterized through the years providing a better understanding of the plant-microbe interactions [14], including the tomato R protein Cf-4 mediating the recognition of the *Cladospodium fulvum* effector protein Avr4 [15,16], the potato R protein R3a that recognises Avr3a effector from *Phytophthora infestans* [17] and the recognition of AvrPto from *Pseudomonas syringae* pv tomato by receptor kinase Pto in tomato [18].

R-mediated resistance is indirectly mediated by PTMs, where resistance (R)-type proteins, such as SNC1, a TIR-NBS-LRR class disease resistance protein, interact with the SUMO targets Topless-related 1 and HDA19, a transcriptional co-repressor and histone deacetylase respectively [11]. Furthermore, SIZ1, a SUMO E3 ligase, negatively regulates salicylic acid (SA) and PAD4-mediated R-mediated gene signalling and *siz1* mutant Arabidopsis plants constitutively express systemic-acquired resistance (SAR) conferring resistance to the

bacterial pathogen *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 expressing avrRps4 [19]. This clearly shows an involvement of PTMs, apart from basal resistance, in induced resistance (IR) defence mechanism, which can potentially be further exploited to fine-tune plant immune system in response to elicitor molecules.

2. Novel roles of post-translational modifications in defence priming

Until recently, plant defence mechanisms were explained based on basal immune responses after pathogen challenge. As stated above, basal resistance, in many cases, is not enough for survival and leads to disease and ultimately a premature death of the host. However, plants are capable of defending themselves and fight off pathogen attack through constitutive and inducible defence mechanisms [20].

Elicitor molecules can induce resistance in plants, and subsequently can enhance the plant basal resistance after perception of elicitor signals against pathogen attack [21]. One of the main mechanisms of induced resistance is priming [22, 23], which enables the plant to fine-tuning its defences for a more rapid and/or more robust response to abiotic and/or biotic stress [24, 25] and implies activation of systemic responses only when the pathogen reaches the infection site [24].

The priming process goes under three phases, which are 1) a pre-priming stimulus or 'naïve' phase, followed by 2) a post-priming stimulus or 'primed phase' (Figure 2) [26, 27, 28] which leads to transcriptional, post-translational, metabolic, physiological and epigenetic re-programming [29], such as DNA methylation and histone modification changes; these changes in chromatin can be mediated by PTMs of histones (H), such as trimethylation of histone 3 at lysine 4 (H3K4me3). The elicitor Benzo(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH)-induced histones 3 and 4 methylation and acetylation of WRKY29, WRKY53 and WRKY6 promoters [28]; histone variants in mammalian cells, such as phosphorylation and ubiquitination [30] and the histone variant H2A.Z is subject to a variety of post-translational modifications, including acetylation, ubiquitination, and SUMOylation [31]; interestingly in Arabidopsis the accumulation of histone H2A substitute H2A.Z has been proposed to be involved in priming suppressed SA-responsive loci (SArlc), such as PR-1, to be 'ready' for transcription [32]. This may provide a link to a 'post-primed phase' where the plant shows an enhanced resistance to pathogen challenge, mainly by a faster and/or stronger defence response [27, 29]. However, the molecular-basis of the linkage between some of the previous changes, in particular post-translational modifications (PTMs), such as protein phosphorylation, ubiquitination, SUMOylation and the more recent lipid-based PTMs and defence priming still remains unclear, however some evidence has been shown such as in

Arabidopsis the *ots1-ots2* double mutant and *siz1* mutant show constitutive SAR and resistance against Pst. DC3000 [19, 33]. Finally, 3) the 'post-primed state' has been related to an increased, more efficient activation of the plant defence response against pathogen attack (Figure 2) with minimal plant fitness costs [34, 35]. Moreover, the 'post-primed state' of the plant results from an amplified sensitization or perception (increased 'alertness') of immunity-inducing signals, rather than from direct gene induction [24, 36], which reinforces the importance of PTMs in the primed cell proteome to "fight back" against biotic and/or abiotic stresses.

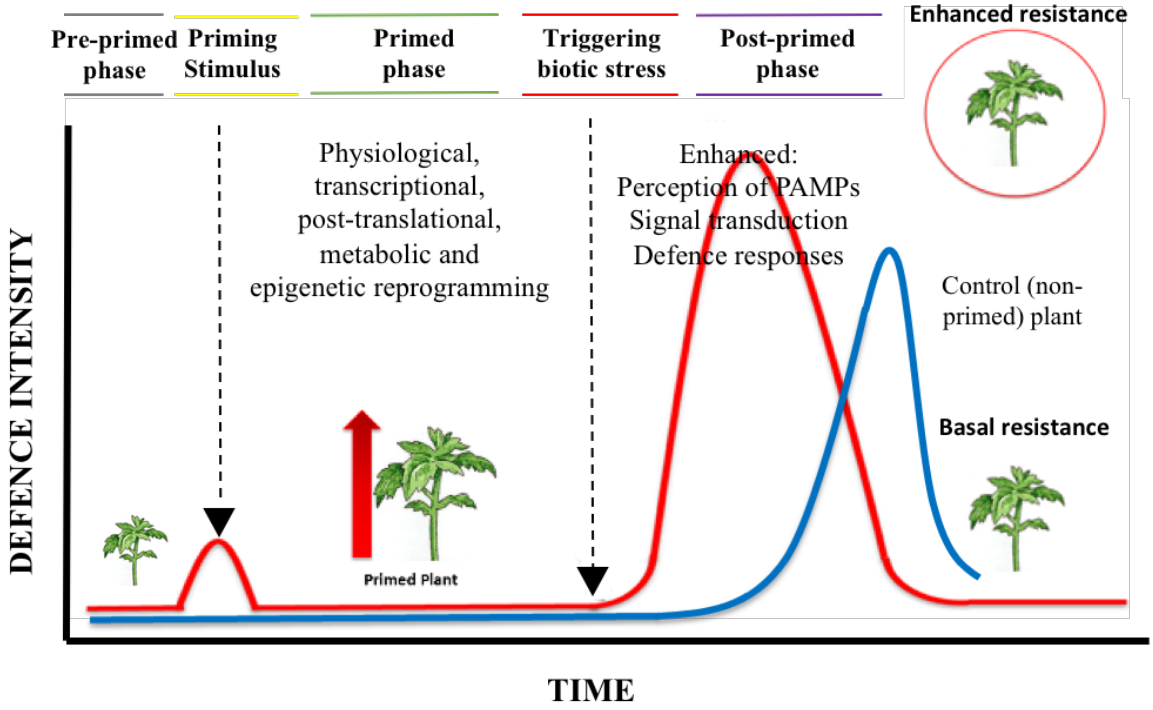

Figure 1. Model of a general priming process with an elicitor or 'priming agent' (adapted from Martinez-Medina et al. 2016). The priming stimulus (e.g. chemical priming agent such as BABA, JA or chitosan) acts on a pre-primed organism which leads to a 'primed phase' and precedes the stress response induced by a triggering stimulus, such as pathogen infection. After the stress trigger (e.g. pathogen attack), the 'post-primed' plant shows a stronger and more rapid defence response which leads to an enhanced resistance against different stresses. The amplitude of defence is shown on the y axis and the time on the x axis.

As stated above, the implications of defence priming are numerous; including long-lasting resistance, changes in transcriptional, post-translational, metabolic and physiological regulation and even transgenerational primed progeny [34]. Some examples include the non-protein amino acid priming elicitor β-aminobutyric acid (BABA), which can induce resistance even 28 days after treatment, termed long-lasting resistance, in *Arabidopsis*

157 *thaliana* against *Hyaloperonospora arabidopsidis* (Hpa) and its priming effect can still be
158 detected in the next generation, which requires the central transcriptional regulator of basal
159 and systemic acquired resistance (SAR) protein NPR1 [37, 38]. The phytohormone jasmonic
160 acid (JA), together with BABA, applied as a seed treatment in tomato, is also able to induce
161 long-lasting priming against herbivores and powdery mildew (*Oidium neolycopersici*) at 8-9
162 weeks after treatment [34] or against *B. cinerea* [21].
163 However, both priming agents, JA and BABA also have an impact on plant growth at high
164 concentrations which must be taken into consideration in order to not over-stress the plant.
165 Even though priming rarely provides complete resistance in the host against biotic stress and
166 it is associated with plant fitness costs and trades-off [22, 39, 40, 41], its benefit relies on the
167 activation of MAMP/DAMP-mediated multi-genic defence response [37] that cannot be
168 easily overcome by the pathogen.
169 Thus, to achieve a more efficient defence strategy that is less costly in terms of plant fitness,
170 it is important, when using priming agents, to assess the effect of the concentration not only
171 on the activation of plant endogenous defences, but also on the growth and stress tolerance of
172 the plant.

173 ## 3. Priming via post-translational modifications as a key regulatory system for the
174 ## onset, speed and outcome of the plant defence response against biotic stress

175 As described above, the three major PTMs, protein phosphorylation, ubiquitination and
176 SUMOylation have been well-established as being key in plant signalling. It has recently
177 been showed that PTMs are essential regulatory mechanisms that enable host cells to deploy
178 defence responses quickly upon pathogen challenge and they can also be targeted by
179 pathogen effectors [10]. Even though the molecular basis of PTMs role in plant defence
180 priming is still largely unknown, several studies have acknowledged the importance of
181 histone acetylation and methylation and transcription factor phosphorylation for the cell to
182 acquire memory by storing information of PTM-induced changes and thus respond faster and
183 more robustly towards the same type of stress subsequently [22, 25, 28, 36].
184 It has also been hypothesized [24] and recently demonstrated [28] that some priming agents,
185 such as BABA, BTH and arbuscular mycorrhiza fungi (AMF), are able to transiently and/or
186 constitutively induce accumulation of cellular molecules, such as mRNAs, reactive oxygen
187 species (ROS), secondary metabolites and hence induce the increase in protein levels, which
188 in turn enhances the signalling component of the cellular immunity mechanisms. This process
189 leads to a more rapid and stronger defence response when the pathogen reaches the primed

cells [23]. It has been hypothesised that the increased abundance of "inactive" immune signalling regulators in primed cells can be linked to PTMs [32], such as protein phosphorylation, ubiquitination and SUMOylation. For example, it has been previously stated that priming agents, such as the SA functional analogue and SAR activator benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) has been reported to prime *A. thaliana* cells by increasing the amount of mitogen-activated protein kinases (MAPK) [25].

MAPK-mediated phosphorylation is a good example of the PTM machinery, as they are both a target and a product for PTM. As noted in Section 1, phosphorylation dynamics are pivotal for MAMP/PAMP perception and PTI and thus for rapid alterations of signalling pathways. However, they can be pathogen targets to deploy infection also, such as the bacterial type III effector proteins from *Pst* DC3000 that targets ROS and MAPK phosphorylation cascades [42]. Interestingly, this suggests a potential link between priming and phosphorylation, as after PAMP perception, the immune signalling cascade is transduced by MPK target phosphorylation. Therefore, there is potential for manipulating the phosphorylation status of MPKs as well as their substrates for defence priming.

In this case, PAMP/MAMP-based priming elicitors, such as *flg22* and chitin-based elicitors, could have an impact on phosphorylation dynamics by activating the expression of defence-related regulatory gene cascades, such as mitogen-activated protein kinases (MPKs) and subsequent MPK kinases (MEKKs), which are involved in signal transduction and promoters of transcription co-activator genes such as the WRKY domain proteins [25], thus significantly increasing the speed of the defence response and improving plant-pathogen interaction outcomes in favour of the host.

Ubiquitination has been commonly associated with protein degradation, protein function regulation and modulation of plant responses to biotic stress [43]. The plant ubiquitin-proteasome system (UPS) is involved in plant growth, development, abiotic stress responses and ultimately plant immunity [44]. Ubiquitin E3 ligases are triggered in response to PAMP-based elicitors and effectors [44] and ubiquitination of defence-related genes is essential for their function, such as the SAR regulatory protein, NPR1, which is translocated into the nucleus via the UPS [44].

Signalling-based genes, such as some Avr9/Cf-9 rapidly elicited (ACRE) genes encode components of signalling cascades, including transcription factors, protein kinases, and ubiquitination pathway-related proteins, such as, E3 ligases, F-box and U-box proteins [43]. Thus, targeting priming plant ubiquitination/UPS opens new possibilities to increase the

speed and efficacy of the plant signalling upon pathogen attack. However, the challenge is to prime the ubiquitin system towards immunity without having an impact in other ubiquitin-related processes, such as plant growth and development.

The role of SUMOylation in disease resistance is an emerging area of importance (Figure 2) where Arabidopsis SUMO E3 ligase (SIZ1) acts as a negative regulator of SA- and PAD4-mediated signalling in plants against Pst DC3000 expressing avrRps4 [19]. Moreover, the importance of SUMO conjugation in plant survival under abiotic stress has been described recently [45]. SUMO conjugation has also been shown to be required to supress defence signalling in the absence of infection [42]. The question then remains as whether SUMOylation and other PTMs can be primed in order to facilitate a rapid immune response to prevent a lethal outcome from disease and lead to resistance.

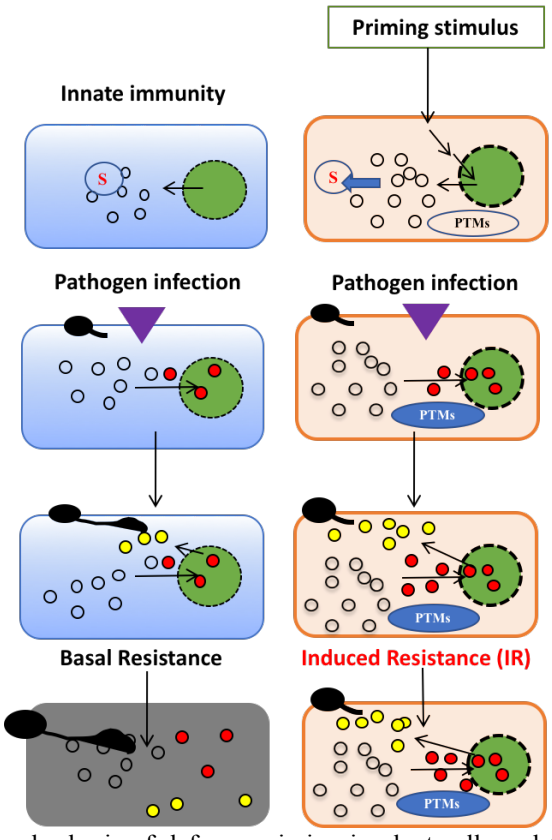

Figure 2. Model of the molecular basis of defence priming in plant cells and the connection to PTMs. In the non-primed (left) cell, the plant cell through the nucleus (green circles) remains with basal expression of defence-related genes and SUMO conjugation (S) represses signalling in pathogen absence. On the primed cell (right) the priming stimulus induces the nuclear-mediated transcription of mRNA, cleavage of SUMO proteins and accumulation of inactive post-translationally modified (PTMs) defence-related proteins. After pathogen challenge both cells trigger expression of signalling cascades and defence-related proteins, however only primed cells are able to quickly translate and activate the defence-related proteins (red circles) that were modified post-

translationally and hence ultimately express a fine-tuned faster defence response that enables the plant to display antimicrobial proteins (yellow circles) that reduce and/or stop pathogen expansion, whereas non-primed cells are not able to display quick defence response which leads into infection expansion and disease.

Interestingly, it has been shown that NPR1 is a SUMO protein target upon salicylic acid (SA) induction and that NPR1 SUMOylation by SUMO3 is required for its immune activity and degradation [38]. This clearly shows the potential implications and connection of PTMs and priming, which is yet to be exploited. It may be possible to find novel PTMs targets, such as JA-dependent transcription factors, e.g. JAZ and MYC2 are well -known PTM targets, which would open up multiple implications for PTMs and long-lasting priming against necrotrophic pathogens.

Few studies have examined this in detail in crop systems. [21] Luna et al., 2016 showed that a soil drench of BABA at high concentrations (10 mM) and JA (1 mM) on 1-week-old tomato seedlings abolished plant growth and had lethal effects. The importance of the SUMO proteases OTS1 and OTS2 has been shown in promoting plant growth under salt stress and that SUMO1 over-expression has a repressive effect on plant development [46]. Thus, it would be interesting to investigate further the molecular basis of this common phenotype of BABA/JA-induced and SUMO1-overexpression related repression of plant development. Furthermore, ots1 ots2 double mutant has been shown to be more resistant against Pst DC3000, hence there may be opportunities to exploit these putative common pathways to boost defence priming and promote growth under stress.

It is well-known that a previous stress stimulus can induce epigenetic changes in the plant and subsequently enhance its defence mechanisms [28]. Moreover, the link between post-translational modifications (PTMs) and priming has been demonstrated through post-translational modifications of histones at promoter regions of primed defence genes [37]; it has also been shown that RNA Polymerase V mutants were enriched in H3K4me3 at the promoter of PR-1 and PDF1.2 defence-related genes, which lead to an enhanced resistance to Pst [47]. Furthermore, application of the hormone salicylic acid (SA) and Pst DC3000 infection has been linked to the accumulation of the acetylated and methylated versions of histones H3 (H3Ac), H4 (H4Ac) and H3K4me2, H3K4me3 and HDA19 at the promoter region of PR-1, WRKY38 and WRKY62. It is postulated that this remodelling of chromatin of these SA-responsive loci may be repressed by SUMO but not shown [32] and therefore it is likely that SUMO will have a critical role in defence priming.

Conclusions and Perspectives

In a world where human population has increased exponentially in recent decades reaching 7.6 billion in 2017 and projected to reach 8.6 billion by 2030 (United Nations, The 2017 Revision of World Population Prospects), a major challenge in the fight against pathogen damage to crop yields worldwide is the ineffectiveness of conventional crop protectants due to pathogen resistance and the fast evolution of pathogens towards their hosts to overcome resistance and promote disease.

Priming has emerged over the last decade as a promising wide-ranging inducible defence mechanism with minimal costs in plant development. Multiple examples have shown the ability of certain molecules to potentiate the plant 'alertness' to perceive and subsequently respond to pathogen attack. The three major post-translational modifications, including phosphorylation, ubiquitination and SUMOylation are key components of the plant immune system, cell signalling and they are inter-linked but their roles in defence priming have yet to be deciphered. Other PTMs such as S-nitrosylation of proteins, irreversible tyrosine nitration, acetylation and methylation have emerged as pivotal mechanisms in the plant immune system with the potential to be primed. Furthermore, there are still many questions as to how these signals are transmitted intra- and even inter-cellularly? How do primed cells regulate post-translational modifications? Are PTMs essential for the establishment of elicitor-induced resistance? What are the molecular mechanisms underlying the priming-related PTMs linked to the fine-tuning and accelerating plant defence responses after pathogen challenge?

Thus, the potential exploitation of PTMs as priming targets has become a 'hotspot' in the race to find new insights in plant immune responses against biotic/abiotic stresses [32] and the current availability of appropriate molecular tools will facilitate deciphering the PTM code for defence priming.

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